

Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer

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Abstract

Objective: The hormone 1,25-dihydroxyvitamin D (1,25(OH)₂D) promotes prostate epithelial cell differentiation *in vitro* and thus, several groups have hypothesized that men who systemically have lower levels of 1,25(OH)₂D may be at increased risk for prostate cancer. To address this hypothesis, we evaluated the association of circulating concentrations of 1,25(OH)₂D and its precursor 25-hydroxyvitamin D (25(OH)D) with subsequent risk of prostate cancer.

Methods: Prostate cancer cases were 460 men in the Health Professionals Follow-up Study who were diagnosed through 1998 after providing a blood specimen in 1993/95. 90.2% of the cases were organ confined or had minimal extraprostatic extension. An equal number of controls who had had a screening PSA test after blood draw were individually matched to cases on age, history of a PSA test before blood draw, and time of day, season, and year of blood draw. Plasma 1,25(OH)₂D and 25(OH)D concentrations were determined by radio-immunosorbant assay blindly to case-control status. Odds ratios (OR) of prostate cancer and 95% confidence intervals (CI) were estimated from conditional logistic regression models mutually adjusting for quartiles of 1,25(OH)₂D and 25(OH)D concentrations and for suspected prostate cancer risk factors. Quartile cutpoints were determined separately by season of blood draw using the distributions among controls.

Results: Mean concentrations of 1,25(OH)₂D and 25(OH)D were slightly, but not statistically significantly ($p = 0.06$ and 0.20 , respectively), higher in cases (34.3 ± 7.1 pg/ml and 24.6 ± 7.7 ng/ml, respectively) than in controls (33.5 ± 7.1 pg/ml and 23.9 ± 8.2 ng/ml, respectively). The OR of prostate cancer comparing men in the top to bottom quartile of 1,25(OH)₂D was 1.25 (95% CI: 0.82–1.90, p -trend = 0.16). For 25(OH)D the OR of prostate cancer comparing the top and bottom quartiles was 1.19 (95% CI: 0.79–1.79, p -trend = 0.59). These findings did not vary by level of the other metabolite, age at diagnosis, family history of prostate cancer, or factors that are thought to influence 25(OH)D levels.

Conclusion: In this prospective study, we did not observe an inverse association between plasma concentrations of 1,25(OH)₂D or 25(OH)D and incident prostate cancer, although we cannot rule out potential effects at later stages of the disease.

Introduction

Schwartz and Hulka put forth the hypothesis that vitamin D protects against prostate cancer based on a correlational study showing that US states with higher ultraviolet radiation tend to have lower prostate cancer

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mortality rates [1]. Subsequently, five case-control studies nested in prospective cohort studies have examined the vitamin D and prostate cancer hypothesis by measuring circulating vitamin D metabolites [2–6]. Only one of these studies supports an inverse association for 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$) [2] and another supports an inverse association for 25-hydroxyvitamin D ($25(\text{OH})\text{D}$) [6].

Vitamin D, or cholecalciferol, is derived mainly from ultraviolet light conversion of 7-dehydrocholesterol in the skin, but also from intake of foods, such as fish, eggs, butter, and fortified milk products and breakfast cereals, and vitamin D-containing multivitamins and supplements. Plasma $25(\text{OH})\text{D}$, formed in the liver from cholecalciferol, varies with exposure to sunlight and intake of vitamin D and is the best indicator of nutritional vitamin D status [7]. The hormone $1,25(\text{OH})_2\text{D}$, produced from $25(\text{OH})\text{D}$ in the kidney, enhances calcium absorption from the small intestine and is tightly regulated to ensure calcium homeostasis [8]. In addition to the main function of this hormone, $1,25(\text{OH})_2\text{D}$ is thought to mediate epithelial cell growth control, including prostate epithelial cells [9–13].

Previously we observed that high calcium intake, in particular at levels achievable primarily through use of calcium supplements, was associated with an increased risk of metastatic prostate cancer [14]. Because high calcium intake suppresses production of $1,25(\text{OH})_2\text{D}$, we hypothesized that men who systemically have lower levels of $1,25(\text{OH})_2\text{D}$ may be at increased risk for prostate cancer. Thus, to address a possible pathway underlying the direct association between high levels of calcium intake and prostate cancer risk in this cohort, we evaluated the association of plasma concentrations of $1,25(\text{OH})_2\text{D}$ and its precursor $25(\text{OH})\text{D}$ with subsequent prostate cancer risk in a case-control study nested in the prospective Health Professionals Follow-up Study. This study consisted of double the number of prostate cancer cases than were included in any of the previous studies. In addition, because an extensive dietary and vitamin supplement history has been collected for these men during cohort follow-up, we were able to consider the potentially modifying effects of these factors.

Materials and methods

Study population

Incident prostate cancer cases and controls were identified among members of the Health Professionals Follow-up Study, an ongoing prospective cohort study

of 51,529 US men who were aged 40–75 years at enrollment in 1986. At baseline the men completed a mailed questionnaire on demographics, lifestyle, and medical history and a semiquantitative food frequency questionnaire. Updated exposure and disease information was collected biennially and diet was collected every 4 years. Deaths were identified through reports by family members or by the postal system in response to the follow-up questionnaires, or were identified through a search of the National Death Index [15]. In 1993–1995, 18,018 of the men provided a blood specimen. The blood was collected in tubes containing sodium EDTA and was shipped by overnight courier while chilled. The blood was centrifuged, aliquotted into plasma, erythrocytes, and buffy coat and stored in liquid nitrogen freezers. We excluded from the analysis men who had a diagnosis of any cancer, except nonmelanoma skin cancer, prior to the date of blood draw. The Human Subjects Committee at the Harvard School of Public Health approved this study.

Prostate cancer cases and controls

For each man who reported a prostate cancer diagnosis on a follow-up questionnaire or for whom a death certificate indicated that the cause of death was prostate cancer, we obtained medical and pathology records after receiving written permission from the participants or their next-of-kin. The response rate was 96% for nonfatal cases and we estimate having ascertained more than 98% of fatal cases. Medical records and pathology reports were successfully obtained for 90% of the cases in the cohort. The remainder of cases included in the analysis was self-reported. A study investigator blinded to exposure information reviewed the records to confirm adenocarcinoma of the prostate and to abstract stage and Gleason sum of the tumor. We classified cases by stage and Gleason sum. We also classified cases as less aggressive (T1b, T1c, T2a, T2b, T3a and N0M0 and Gleason sum <7) and more aggressive (T3bN0M0, T4N0M0, any T N1, any T M1 or Gleason sum ≥ 7) disease. We included tumors of unknown stage and grade along with less aggressive cases. We excluded incidental microscopic focal tumors (T1a) from the analysis because these tumors are generally indolent and are most susceptible to detection bias due to differential rates of undergoing surgery for benign prostatic hyperplasia. We also excluded three cases because a date of diagnosis could not be established. We confirmed 463 non-T1a prostate cancer cases between the date of blood draw and January 31, 1998.

To be eligible to be selected as a control, a man must have had a PSA test after the date of blood draw to have

the opportunity to have an occult prostate cancer diagnosed. For each case, 1 control was selected who was still alive and did not have a diagnosis of cancer by the date of the case's prostate cancer diagnosis. Controls were matched to cases on year of birth (± 1 year), PSA test prior to blood draw (yes/no), and timing of blood draw – time of day (midnight to before 9 am, 9 am to before noon, noon to before 4 pm, 4 pm to before midnight), season (winter, spring, summer, fall), and year (exact). Ninety-two percent of the controls had a PSA test 2.5 years or less before their matched case's date of diagnosis.

Vitamin D assays

Plasma concentrations of 1,25(OH)₂D and 25(OH)D were determined by radioimmunosorbant assay (RIA) in the laboratory of Dr. Bruce Hollis as described previously [7, 16]. Cases diagnosed from the date of blood draw through January 1996 and their matched pairs and cases diagnosed from February 1996 through January 1998 and their matched pairs were assayed in two separate analytical runs. We did not use one case–control set because the volume of remaining plasma was low. In addition, 1,25(OH)₂D concentrations were not determined for one case and one control. We excluded these sets, leaving 460 complete case–control pairs for the statistical analysis. The mean intrapair coefficients of variation calculated from blinded quality control samples were 5.3% for 1,25(OH)₂D and 5.4% for 25(OH)D in the first set and 7.3% for 1,25(OH)₂D and 5.6% for 25(OH)D in the second set. To assess the intraperson consistency of vitamin D metabolite concentrations over time, we measured 1,25(OH)₂D and 25(OH)D for 144 men in the Health Professionals Follow-up Study cohort who were free of a cancer diagnosis and who provided a blood sample in 1993/94 and again in 1997 (mean of 3.03 ± 0.46 years apart). Adjusting for age, race, and season of the year, the Pearson correlation coefficients between the two time points were 0.50 ($p < 0.0001$) for 1,25(OH)₂D and 0.70 ($p < 0.0001$) for 25(OH)D. The lower intraperson correlation for 1,25(OH)₂D compared to 25(OH)D is likely due to restricted inter-person variance for 1,25(OH)₂D because of physiologic conservation of 1,25(OH)₂D, but not 25(OH)D, between individuals.

Statistical analysis

We compared mean concentrations of the vitamin D metabolites and the molar ratio of 1,25(OH)₂D to 25(OH)D, as a measure of the relative conversion, between matched cases and controls using the paired

t-test. We estimated the odds ratio (OR) of prostate cancer and 95% confidence intervals (CI) from conditional logistic regression models with plasma concentrations of 1,25(OH)₂D, 25(OH)D, and their molar ratio entered into the models as a series of indicator variables for quartiles. Because the samples were assayed in two analytical runs and because vitamin D levels vary by season of the year, we used separate quartile cutpoints defined by analytical run and season of the year and based on the distributions among the controls. In addition to running models that took into account the matching factors, we ran multivariate models mutually adjusting for the vitamin D metabolites and for the following known or suspected prostate cancer risk or protective factors: father or brother with prostate cancer (1990 – yes/no), height (1986 – continuous), vigorous physical activity (continuous), diabetes mellitus (yes/no), vasectomy (yes/no), cigarette smoking in the past 10 years (current, former), intake (continuous) of energy, red meat, and fish, intake (continuous) of energy-adjusted lycopene (1990), fructose (1990), and α -linolenic acid, and use (yes/no) of vitamin E and selenium supplements. We used covariate values from the 1994 follow-up questionnaire, the one closest in time to blood draw, except where specified. Values from 1990 or 1986 were substituted for information missing in 1994. In the main analysis, we did not adjust for calcium intake, which is positively associated with advanced disease in this cohort, because one potential mechanism underlying the effect of calcium is the reduction in systemic 1,25(OH)₂D levels. To test for trend, we entered into the model a single ordinal variable with values of 1–4 corresponding to the quartile in which an individual's plasma vitamin D metabolite level fell. We separately estimated the ORs of more aggressive and less aggressive disease. Other classifications of disease aggressiveness defined by stage and Gleason sum were also evaluated. We estimated the OR of total prostate cancer for a plasma 1,25(OH)₂D concentration below 26 pg/ml [17] or for a plasma 25(OH)D concentration below 15 ng/ml [18], levels typically considered to be clinically below normal, irrespective of season.

To evaluate whether the association between circulating vitamin D metabolites and prostate cancer varied by level of the other metabolite, family history of prostate cancer, or determinants of 25(OH)D concentration, we ran unconditional logistic regression models adjusting for the matching factors and the suspected prostate cancer risk factors and stratified by level of the other metabolite (median or tertiles), father or brother with prostate cancer (yes/no), intake of total dietary and supplemental calcium (*>versus* ≤ 1000 mg/day), total dietary and supplemental vitamin D

(>versus ≤ 400 IU/day), total dairy products (>versus ≤ 13 servings/week), and milk as a beverage (>versus ≤ 5.5 servings/week), and region of the country of residence as a marker for extent of exposure to ultraviolet light (Northeast, South, Midwest, West). To determine if these associations varied by the matching factors age at diagnosis or season of the year of blood draw, we ran the multivariate conditional logistic regression models stratified as <64 (25th percentile) and ≥ 64 years old at diagnosis and as winter/spring and summer/fall. To test for statistical interaction, we entered into the appropriate multivariate model the main effect terms for the metabolite and the covariate along with a term for the product of the covariate and metabolite, the coefficient for which was evaluated by the Wald test. All analyses were conducted using SAS release 8.2 (SAS Institute, Cary, NC). We report two-sided *p*-values for all hypothesis tests.

Results

The mean age at prostate cancer diagnosis was 68.7 years old (range 47.8–84.3 years). Of the 84% of cases for whom information was available, 90.2% were organ confined (T1b, T1c, T2a or T2b) or had minimal extraprostatic extension (T3a) and the most common Gleason sums were 6 (33.1%), 7 (29.2%), and 5 (19.1%). For the 63.7% of cases for whom diagnostic PSA was available, the median was 7.0 ng/ml and the mean was 12.0 ± 24.6 ng/ml. Mean time between blood draw and diagnosis was 2.2 ± 1.2 years. Prostate cancer cases and their matched controls did not notably differ on suspected risk factors for prostate cancer, with the possible exception of use of a vitamin E supplement, or on characteristics that might influence vitamin D metabolite concentrations (Table 1). The vast majority of the cases and the controls were white. At least one screening PSA test prior to blood draw was reported by 78.7% of cases and 79.6% of controls ($p = 0.68$).

Mean plasma concentrations of $1,25(\text{OH})_2\text{D}$ and $25(\text{OH})\text{D}$ were slightly, but not statistically significantly ($p = 0.06$ and 0.20 , respectively), higher in cases (34.3 ± 7.1 and 24.6 ± 7.7 ng/ml, respectively) than in controls (33.5 ± 7.1 and 23.9 ± 8.2 ng/ml) (Table 2). No difference in the molar ratio of $1,25(\text{OH})_2\text{D}$ to $25(\text{OH})\text{D}$ was seen. A plasma concentration of $25(\text{OH})\text{D}$ below 15 ng/ml, which is compatible with clinical vitamin D deficiency, was present in 6.1% of cases and 11.3% of controls. 10.4% of cases and 11.1% of controls had $1,25(\text{OH})_2\text{D}$ concentrations below normal (<26 pg/ml).

Compared to the lowest quartile of $1,25(\text{OH})_2\text{D}$ the ORs of prostate cancer for the top three quartiles were 0.98, 1.08, and 1.35. The ORs for the top three quartiles of $25(\text{OH})\text{D}$ compared to the bottom quartile were 1.07, 0.83, and 1.34. No association was observed for the molar ratio of $1,25(\text{OH})_2\text{D}$ to $25(\text{OH})\text{D}$ (Table 3). To evaluate the independent effects of $1,25(\text{OH})_2\text{D}$ and $25(\text{OH})\text{D}$, which were weakly correlated (among the controls: Pearson $r = 0.25$, $p < 0.0001$), we mutually adjusted for the two vitamin D metabolites. The OR in the highest versus lowest quartiles of $1,25(\text{OH})_2\text{D}$ (OR = 1.25) and $25(\text{OH})\text{D}$ (OR = 1.27) were slightly attenuated (Table 3). After adjusting for suspected risk factors for prostate cancer the ORs for the highest versus lowest quartiles were 1.25 (95% CI: 0.82–1.90) and 1.19 (95% CI: 0.79–1.79), for $1,25(\text{OH})_2\text{D}$ and $25(\text{OH})\text{D}$, respectively (Table 3). The results were similar when excluding men whose prostate cancer was diagnosed within 2 years of blood draw (not shown).

Findings for less aggressive disease were similar to overall prostate cancer (Table 4). For more aggressive disease, there was no association in models that took into account only the matching variables, but after adjustment for suspected prostate cancer risk factors the ORs for the top two quartiles of both metabolites were below 1. Neither was statistically significant and there was no trend (Table 4). The ORs (taking into account matching factors only) of stage T3b or worse prostate cancer ($n = 40$ cases) comparing the top quartiles of $1,25(\text{OH})_2\text{D}$ and $25(\text{OH})\text{D}$ to the bottom were 0.19 (p -trend = 0.16) and 0.37 (p -trend = 0.44), respectively. However, neither of these estimates nor the trends across quartiles was statistically significant. In general, no clear patterns emerged when considering other categorizations to define aggressive versus nonaggressive disease, including other categories of stage, other combinations of stage and grade, and excluding those with missing stage and grade information from the less aggressive cases (not shown).

After adjusting for $25(\text{OH})\text{D}$ and the suspected prostate cancer risk factors, having clinically low $1,25(\text{OH})_2\text{D}$ concentration (<26 pg/ml) was not associated with prostate cancer (OR = 0.98, 95% CI: 0.62–1.56) overall or after breaking the matched pairs, among men whose $25(\text{OH})\text{D}$ concentration was below (OR = 0.90, 95% CI: 0.51–1.58) or at or above (OR = 0.93, 95% CI: 0.46–1.92) the median. The adjusted OR of prostate cancer for men with a clinically low plasma $25(\text{OH})\text{D}$ concentration (<15 ng/ml) compared to other men was 0.49 (95% CI: 0.28–0.83). The inverse association was observed both for men below (OR = 0.48, 95% CI: 0.24–0.93) and at or above (OR = 0.57, 95% CI: 0.25–1.27) the median

Table 1. Characteristics^a of 460 prostate cancer cases and 460 matched controls nested in the Health Professionals Follow-up Study, 1993–1998

	Cases	Controls	<i>p</i> -Value ^b
Age at blood draw (year)	66.5 ± 7.4	66.4 ± 7.4	Matching variable
Nonwhite (%)	5.4	6.3	0.67
Family history of prostate cancer (%)	14.1	11.3	0.22
Height in 1986 (in)	70.1 ± 2.8	70.1 ± 2.6	0.86
Body mass index at age 21 (kg/m ²)	22.8 ± 2.8	23.0 ± 2.8	0.30
Current body mass index (kg/m ²)	26.0 ± 3.8	26.0 ± 3.5	0.81
Vigorous physical activity (MET – h/week)	11.6 ± 23.6	12.1 ± 24.3	0.76
Cigarette smoker in the past 10 years (%)	18.0	17.4	0.85
Diabetes mellitus (%)	3.9	3.2	0.71
Vasectomy (%)	26.1	26.3	1.00
Supplement user			
Vitamin E (%)	38.3	32.8	0.10
Selenium (%)	7.6	8.3	0.81
Calcium (%)	19.1	17.2	0.51
Energy-adjusted mean intake			
Calcium (mg/day)	975 ± 474	950 ± 455	0.60
Vitamin D (IU/day)	459 ± 268	436 ± 271	0.31
Fructose in 1990 (g/day)	49.8 ± 14.7	49.3 ± 16.4	0.64
Lycopene in 1990 (μg/day)	8946 ± 6475	9533 ± 7009	0.19
α-linolenic acid (g/day)	1.05 ± 0.28	1.05 ± 0.29	0.97
Energy intake (kcal/day)	2007 ± 573	2022 ± 627	0.70
Dairy intake (servings/week)	14.5 ± 8.7	14.0 ± 8.1	0.40
Red meat intake (servings/week)	7.2 ± 4.5	7.2 ± 5.2	0.87
Fish intake (servings/week)	2.1 ± 1.5	2.3 ± 1.6	0.17
Season during which blood was drawn			Matching variable
Winter	17.0	15.2	
Spring	20.7	20.4	
Summer	29.8	30.2	
Fall	32.6	34.1	
Region of the country of residence			
Northeast (%)	17.4	17.4	0.74
South (%)	30.9	29.6	
Midwest (%)	25.9	26.5	
West (%)	25.9	26.1	
Other region (%)	0.0	0.4	

^a Unless otherwise indicated, values are from the 1994 follow-up questionnaire. Dairy, red meat, and fish intake are averages of reports in 1986, 1990, and 1994.

^b For the hypothesis test of no difference in means (paired *t*-test) or proportions (McNemar's test) between prostate cancer cases and controls. All tests are 2-sided.

concentration of 1,25(OH)₂D. Five cases and six controls had clinically low levels of both vitamin D metabolites; risk was not increased in these men (OR = 0.81, 95% CI: 0.24–2.75).

Table 2. Plasma concentrations of 1,25(OH)₂D and 25(OH)D in prostate cancer cases and matched controls nested in the Health Professionals Follow-up Study, 1993–1998

	Cases	Controls	<i>p</i> -Value ^a
No	460	460	
Plasma concentration			
1,25(OH) ₂ D (pg/ml)	34.3 ± 7.1	33.5 ± 7.1	0.06
25(OH)D (ng/ml)	24.6 ± 7.7	23.9 ± 8.2	0.20
Molar ratio of 1,25(OH) ₂ D to 25(OH)D (×10 ⁻³)	1.53 ± 0.58	1.56 ± 0.67	0.40

^a For the hypothesis test of no difference in means (paired *t*-test) between prostate cancer cases and controls. All tests are 2-sided.

Table 3. Association of plasma 1,25(OH)₂D and 25(OH)D with prostate cancer in 460 matched pairs nested in the Health Professionals Follow-up Study, 1993–1998

	Quartile ^a				<i>p</i> -Trend ^b
	1	2	3	4	
1,25(OH) ₂ D					
No. cases	102	103	120	135	
No. controls	110	115	122	113	
OR ^c	1.00	0.99	1.08	1.25	0.16
95% CI	Referent	0.67–1.45	0.73–1.61	0.83–1.88	
OR ^d	1.00	0.93	1.04	1.25	0.16
95% CI	Referent	0.62–1.39	0.69–1.56	0.82–1.90	
25(OH)D					
No. cases	109	115	94	142	
No. controls	114	113	120	113	
OR ^c	1.00	1.05	0.82	1.27	0.41
95% CI	Referent	0.71–1.54	0.56–1.20	0.86–1.86	
OR ^d	1.00	1.00	0.77	1.19	0.59
95% CI	Referent	0.67–1.49	0.51–1.15	0.79–1.79	
Molar ratio of 1,25(OH) ₂ D to 25(OH)D (×10 ^{−3})					
No. cases	109	123	125	103	
No. controls	119	107	124	110	
OR	1.00	1.26	1.11	1.02	0.92
95% CI	Referent	0.87–1.84	0.77–1.59	0.68–1.52	
OR ^e	1.00	1.32	1.14	1.09	0.85
95% CI	Referent	0.90–1.94	0.78–1.67	0.72–1.66	

^a Medians for each quartile: Among controls matched to cases diagnosed between date of blood draw and 1/1996: 25(OH)D – summer: 20.1, 24.1, 27.6, 32.9; fall: 14.9, 22.4, 25.4, 31.1; spring: 12.0, 16.6, 20.9, 24.5; winter: 9.3, 15.8, 19.9, 24.6. 1,25(OH)₂D – summer: 27.0, 31.0, 35.7, 42.6; fall: 26.8, 30.1, 34.4, 39.9; spring: 25.6, 28.9, 33.7, 37.1; winter: 19.1, 27.1, 33.5, 39.2. Ratio (×10⁻³) – summer: 0.89, 1.25, 1.38, 1.90; fall: 0.99, 1.20, 1.54, 2.11; spring: 1.00, 1.54, 1.88, 2.78; winter: 1.06, 1.42, 1.81, 2.84. Among controls matched to cases diagnosed 2/1996 – 1/1998: 25(OH)D in ng/ml – summer: 17.4, 23.8, 28.4, 36.5; fall: 16.8, 22.0, 27.7, 38.2; spring: 13.9, 19.7, 24.0, 29.9; winter: 13.8, 19.4, 21.8, 26.9. 1,25(OH)₂D in pg/ml – summer: 25.5, 30.6, 37.3, 45.2; fall: 27.5, 31.9, 36.7, 44.0; spring: 25.0, 30.0, 34.3, 38.8; winter: 27.6, 31.7, 36.8, 39.9. Ratio (×10⁻³) – summer: 0.88; 1.11, 1.47, 1.96; fall: 0.95, 1.14, 1.54, 2.10; spring: 0.91, 1.33, 1.56, 2.19; winter: 1.14, 1.50, 1.84, 2.70.

^b From a test for trend in which plasma level was entered into the model as a single ordinal variable with values of 1–4 corresponding to the quartile in which the individual fell.

^c Mutually adjusted for 1,25(OH)₂D and 25(OH)D using conditional logistic regression.

^d Mutually adjusted for 1,25(OH)₂D and 25(OH)D and for family history, height, vigorous physical activity, diabetes mellitus, vasectomy, cigarette smoking in the past 10 years, intake of energy, red meat, fish, lycopene, fructose, and α -linolenic acid, use of vitamin E, and selenium supplements using conditional logistic regression.

^e Adjusted for family history, height, vigorous physical activity, diabetes mellitus, vasectomy, cigarette smoking in the past 10 years, intake of energy, red meat, fish, lycopene, fructose, and α -linolenic acid, use of vitamin E, and selenium supplements using conditional logistic regression.

We also evaluated whether risk of prostate cancer varied by age at diagnosis, family history of prostate cancer, and factors that might influence vitamin D metabolite levels, such as intakes of calcium, vitamin D, milk as a beverage (not shown), and total dairy, region of the country of residence (not shown), or season of the year of blood draw (not shown). There was no consistent evidence of inverse associations between the vitamin D metabolites and prostate cancer risk within strata of these factors (Table 5). Based on very small numbers ($n = 65$ cases), among men with a positive family history of prostate cancer there was a statistically significant increased risk of prostate cancer with higher plasma 25(OH)D concentrations.

Discussion

In this prospective study we did not observe inverse associations between circulating concentrations of 1,25(OH)₂D, 25(OH)D, or their molar ratio and subsequent risk of prostate cancer overall, although we cannot rule out potential effects at later stages of the disease. Inverse associations for 1,25(OH)₂D or 25(OH)D with prostate cancer were not observed within strata of the other vitamin D metabolite, age at diagnosis, family history of prostate cancer, or factors that influence circulating 25(OH)D concentrations. A major strength of this study was that prostate cancer detection bias that was differential by vitamin D

Table 4. Association of plasma 1,25(OH)₂D and 25(OH)D by prostate cancer aggressiveness in 460 matched pairs nested in the Health Professionals Follow-up Study, 1993–1998

	Quartile ^a				<i>p</i> -Trend ^b
	1	2	3	4	
<i>More aggressive disease^c</i>					
1,25(OH) ₂ D					
No. cases	35	40	40	48	
No. controls	38	29	49	47	
OR ^d	1.00	1.49	0.86	1.06	0.89
95% CI	Referent	0.74–2.99	0.42–1.75	0.53–2.15	
OR ^e	1.00	1.24	0.77	0.80	0.45
95% CI	Referent	0.57–2.73	0.33–1.76	0.36–1.82	
25(OH)D					
No. cases	42	46	31	44	
No. controls	40	37	44	42	
OR ^d	1.00	1.29	0.74	1.06	0.60
95% CI	Referent	0.67–2.49	0.40–1.37	0.54–2.07	
OR ^e	1.00	1.14	0.55	0.78	0.18
95% CI	Referent	0.54–2.41	0.28–1.12	0.35–1.73	
<i>Less aggressive disease^f</i>					
1,25(OH) ₂ D					
No. cases	66	63	80	86	
No. controls	71	85	73	66	
OR ^d	1.00	0.80	1.15	1.35	0.09
95% CI	Referent	0.50–1.30	0.71–1.85	0.82–2.25	
OR ^e	1.00	0.75	1.12	1.26	0.16
95% CI	Referent	0.46–1.24	0.67–1.86	0.74–2.16	
25(OH)D					
No. cases	67	69	63	96	
No. controls	74	76	75	70	
OR ^d	1.00	0.94	0.90	1.41	0.17
95% CI	Referent	0.58–1.53	0.55–1.48	0.87–2.26	
OR ^e	1.00	0.90	0.87	1.40	0.19
95% CI	Referent	0.54–1.52	0.51–1.48	0.83–2.35	

^a See Table 3, footnote 1 for medians for each quartile.^b From a test for trend in which plasma level was entered into the model as a single ordinal variable with values of 1–4 corresponding to the quartile in which the individual fell.^c Primary tumor T3b, T4a, T4b or N1 or M1 or death or Gleason sum ≥ 7 .^d Mutually adjusted for 1,25(OH)₂D and 25(OH)D using conditional logistic regression.^e Mutually adjusted for 1,25(OH)₂D and 25(OH)D and adjusted for family history, height, vigorous physical activity, diabetes mellitus, vasectomy, cigarette smoking in the past 10 years, intake of energy, red meat, fish, lycopene, fructose, and α -linolenic acid, use of vitamin E, and selenium supplements using conditional logistic regression.^f Primary tumor T1b, T1c, T2a, T2b, or T3a and N0 and M0 and Gleason sum < 7 .

metabolite status was minimized because we included in the analysis as controls only men who had undergone a screening PSA test.

We evaluated the hypothesis that circulating levels of vitamin D metabolites are associated with the incidence of prostate cancer. Vitamin D is thought to mediate differentiation and growth control of the epithelium in the prostate and other tissues [9–13]. 1,25(OH)₂D transactivates transcription via binding to the cytoplasmic vitamin D receptor [19]. Prostate epithelium expresses functional vitamin D receptors and at physiologic levels 1,25(OH)₂D binds to the vitamin D

receptor in established prostate cancer cell lines and in primary cultures of human prostatic epithelial cells derived from malignant tissue [10, 20–22]. 1,25(OH)₂D has been shown to inhibit proliferation and induce differentiation of prostatic epithelial cells [9–13]. Analogues of 1,25(OH)₂D inhibit growth of human prostate tumor xenografts in nude mice [23] and inhibit growth of tumors in rodents induced by experimental mutagens and promoted by androgens [24].

Dietary and supplemental intake of vitamin D is not associated with prostate cancer in this cohort [14] or elsewhere [25, 26]. In some studies high intake of

Table 5. OR of prostate cancer by quartiles of plasma 1,25(OH)₂D and 25(OH)D stratified by age, family history of prostate cancer, and factors that may influence plasma vitamin D level in 460 matched pairs nested in the Health Professionals Follow-up Study, 1993–1998

	Quartile ^a				<i>p</i> -Trend ^b	<i>p</i> -Interaction ^c
	1	2	3	4		
<i>1,25(OH)₂D (pg/ml)</i>						
Age at diagnosis						
<64 years	1.00	1.27	1.61	1.61	0.17	0.23
≥64 years	1.00	0.82	0.88	1.08	0.55	
Family history						
Negative	1.00	0.96	1.10	1.29	0.17	0.59
Positive	1.00	0.89	1.36	1.18	0.58	
Calcium intake						
>1000 mg/day	1.00	0.89	0.98	1.20	0.45	0.35
≤1000 mg/day	1.00	1.11	1.29	1.52	0.08	
Vitamin D intake						
>400 IU/day	1.00	1.34	1.09	1.32	0.52	0.74
≤400 IU/day	1.00	0.67	1.04	1.23	0.19	
Dairy intake						
>13 servings/week	1.00	1.17	1.62	1.76	0.02	0.16
≤13 servings/week	1.00	0.80	0.75	0.87	0.70	
<i>25(OH)D (ng/ml)</i>						
Age at diagnosis						
<64 years	1.00	1.42	0.92	1.53	0.77	0.34
≥64 years	1.00	0.93	0.71	1.13	0.70	
Family history						
Negative	1.00	1.00	0.71	1.11	0.87	0.07
Positive	1.00	2.92	2.63	7.03	0.02	
Calcium intake						
>1000 mg/day	1.00	0.50	0.43	0.80	0.72	0.43
≤1000 mg/day	1.00	1.23	0.92	1.35	0.41	
Vitamin D intake						
>400 IU/day	1.00	0.81	0.52	0.92	0.58	0.61
≤400 IU/day	1.00	1.13	0.85	1.26	0.49	
Dairy intake						
>13 servings/week	1.00	1.00	0.53	1.32	0.61	0.75
≤13 servings/week	1.00	1.02	1.12	1.09	0.59	

^a See Table 3, footnote 1 for medians for each quartile.

^b From a Wald test for the coefficient for metabolite level, which was entered into the model as a single ordinal variable with values of 1–4 corresponding to the quartile in which the individual fell.

^c From a Wald test for the coefficient for the cross product of the covariate (binary) and the metabolite level (ordinal).

^d Mutually adjusted for 1,25(OH)₂D and 25(OH)D and adjusted for family history, height, vigorous physical activity, diabetes mellitus, vasectomy, cigarette smoking in the past 10 years, intake of energy, red meat, fish, lycopene, fructose, and α -linolenic acid, use of vitamin E, and selenium supplements. Used conditional logistic regression adjusting when stratifying by age at diagnosis. Used logistic regression when stratifying by the other factors, additionally controlling for the matching factors the matching factors age at diagnosis, PSA test prior to blood draw, quadrant of the day of blood draw, season of blood draw.

calcium, generally above reference daily intake, is associated with an increased risk of prostate cancer [14, 25, 26], especially advanced disease [14, 25]. These observations are consistent with high dietary calcium, but not low vitamin D (except possibly in deficiency), down regulating 1,25(OH)₂D production.

Corder *et al.* [2] reported that cases had statistically significantly lower plasma 1,25(OH)₂D concentrations than controls, which was more pronounced in men who were older at study entry (≥57 years). None of the other

prospective studies [3–5], including the present study, observed consistent evidence for an inverse association for 1,25(OH)₂D. The Ahonen *et al.* study [6] did not measure 1,25(OH)₂D, but they did observe a 1.7-times higher risk of prostate cancer (95% CI: 1.2–2.5) in Finnish men whose plasma 25(OH)D concentrations were below the median compared to at or above. The association was strongest in men who were younger at study entry (<52 years old: OR = 3.1, 95% CI: 1.6–6.1) [6]. The other studies were essentially null for 25(OH)D

[2–5]. When considering jointly $1,25(\text{OH})_2\text{D}$ and $25(\text{OH})\text{D}$ levels, reduced risks of prostate cancer were noted for high $1,25(\text{OH})_2\text{D}$ and low $25(\text{OH})\text{D}$ levels in the Corder *et al.* study [2], high levels of both metabolites in the Nomura *et al.* study [5], and possibly high $1,25(\text{OH})_2\text{D}$ with highest or lowest $25(\text{OH})\text{D}$ levels in the Gann *et al.* study [4]. However, the joint association was statistically significant only in the Corder *et al.* study [2]. We did not observe an interaction between levels of the vitamin D metabolites.

The authors of the Finnish study indicated that more than half of their participants had $25(\text{OH})\text{D}$ levels consistent with clinical vitamin D deficiency [6]; their median of 40 nmol/l (16.0 ng/ml) was near the cutpoint for vitamin D deficiency of 15 ng/ml. In the other studies, the percentages of controls that had $25(\text{OH})\text{D}$ lower than 15 ng/ml were ~5% [3], 6.5% [4], 11% (present study), and 13.3% [2]. The proportion with vitamin D deficiency was not available in the Nomura *et al.* study, which was conducted in Hawaii, but it is likely low given that their median $25(\text{OH})\text{D}$ concentration was 41 ng/ml in the cases and 41.6 ng/ml in the controls [5]. Thus, a possible explanation for the inconsistent findings for $25(\text{OH})\text{D}$ and prostate cancer among studies is that in individuals with adequate vitamin D levels there is no added benefit of higher circulating levels, but an incremental increase in $25(\text{OH})\text{D}$ to sufficiency may reduce risk. In the present study clinically low plasma $25(\text{OH})\text{D}$ concentration was inversely associated with prostate cancer. However, this finding was based on only a small number of men with clinically low levels and could represent a chance finding.

Explanations for the lack of consistency in findings for $1,25(\text{OH})_2\text{D}$ and prostate cancer among studies are less clear. In evaluating this relation, we did not simultaneously consider transport proteins, receptors, coactivators, or enzymes involved in vitamin D metabolism, all of which could influence the action of $1,25(\text{OH})_2\text{D}$. Further, circulating $1,25(\text{OH})_2\text{D}$ level may be a poor surrogate for intraprostatic level, as both normal and malignant prostate cell lines express $1-\alpha$ -hydroxylase and can convert $25(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}$ *in vitro* [27]. Thus, those with higher circulating $25(\text{OH})\text{D}$ might be predicted to have higher intraprostatic levels of $1,25(\text{OH})_2\text{D}$ and those with lower circulating $25(\text{OH})\text{D}$ might be predicted to have lower intraprostatic levels of $1,25(\text{OH})_2\text{D}$. However, regulation of the balance of intraprostatic cellular vitamin D metabolites relative to circulating levels is not known. Alternatively, those with high $1,25(\text{OH})_2\text{D}$ and low $25(\text{OH})\text{D}$ may represent those with higher conversion to $1,25(\text{OH})_2\text{D}$ and thus, might be predicted to be at lower

risk. In our study neither men with higher levels of both metabolites or higher $1,25(\text{OH})_2\text{D}$ and lower $25(\text{OH})\text{D}$ levels had a lower risk compared to men with lower levels of both metabolites. However, few of the men in the reference group had very (clinically) low levels of both metabolites, and thus, the variation in intraprostatic levels of $1,25(\text{OH})_2\text{D}$ between men may have been limited.

Another possible explanation for the lack of association of vitamin D metabolites with prostate cancer in this nested case-control study is that the cases were diagnosed during the PSA era in a group of men with a high proportion screened, rather than metastatic cases that were detected because of symptoms. The greatest observable benefit of $1,25(\text{OH})_2\text{D}$ might be against the development of metastatic prostate cancer. This hypothesis is also consistent with high calcium being strongly related to metastatic and fatal prostate cancer, but not with early cases. Evidence supporting a role for protection against prostate cancer metastasis comes from experiments showing that $1,25(\text{OH})_2\text{D}$ decreases the invasiveness of cells derived from prostate cancer metastases likely by reducing expression of certain integrins that play a role in cell adhesion [28]. In the Corder *et al.* [2], Gann *et al.* [4], Ahonen *et al.* [6], studies, all or most cases were ascertained in the pre-PSA era. In each of those studies there was a suggestion that vitamin D metabolites were more strongly inversely associated with more advanced or aggressive disease [2, 4, 6]. With the onset of PSA screening, the spectrum of prostate cancer diagnosed has shifted from clinically detectable (*e.g.*, palpable, symptomatic, metastatic) cases of higher stage to clinically occult early stage disease. In our study the great majority of cases were stages T1b, T1c, T2a, T2b, and T3a. Because vitamin D metabolites are hypothesized to influence differentiation and apoptosis, it is possible that the effect of vitamin D on prostate cancer is not detectable for these early cases. Compatible with this hypothesis, we observed that higher levels of vitamin D metabolites were inversely associated with regionally invasive or regionally/distant metastatic disease, although these findings were based on very few cases ($n = 40$).

This study has several strengths, including that vitamin D metabolites were measured in plasma collected prior to the diagnosis of prostate cancer. Although the mean time between blood draw and diagnosis was 2 years, because the majority of the prostate cancer cases were early stage disease, it is unlikely that yet undetected disease influenced plasma vitamin D metabolite levels, which was supported by an analysis excluding cases diagnosed within the first 2 years after blood draw. The number of cases was

large in the main analysis, which helps to rule out chance variation as an explanation for our null results. The plasma vitamin D metabolites were measured with high precision and sensitivity precluding nondifferential assay measurement error as a contributor to our null findings. Measurement error resulting from measurement of plasma vitamin D metabolites once during middle age is a possibility. However, in a subset of men in this cohort we showed good correlation between two measures of 1,25(OH)₂D and 25(OH)D in blood taken a mean of three years apart, suggesting that at least over the short-term in middle-age one measurement of circulating levels is reasonably representative. We increased the opportunity for equal detection of prostate cancer and reduced the possibility of latent, but yet undiagnosed prostate cancer, in the controls by requiring that controls have had a PSA test after blood draw. In doing so, we limited the likelihood of observation bias that might have resulted if opportunity for disease detection was differential by vitamin D metabolite level.

We conclude that higher circulating levels of 1,25(OH)₂D and 25(OH)D are not associated with a decreased risk of prostate cancer in this cohort, although we cannot rule out potential effects at later stages of the disease.

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